

SHORT

COMMUNICATION

Tamoxifen and its active metabolites inhibit dopamine transporter function independently of the estrogen receptors

Sarah R. Mikelman, Bipasha Guptaroy and Margaret E. Gnegy

*Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan, USA***Abstract**

As one of the primary mechanisms by which dopamine signaling is regulated, the dopamine transporter (DAT) is an attractive pharmacological target for the treatment of diseases based in dopaminergic dysfunction. In this work we demonstrate for the first time that the commonly prescribed breast cancer therapeutic tamoxifen and its major metabolites, 4-hydroxytamoxifen and endoxifen, inhibit DAT function. Tamoxifen inhibits [³H]dopamine uptake into human DAT (hDAT)-N2A cells *via* an uncompetitive or mixed mechanism. Endoxifen, an active metabolite of tamoxifen, asymmetrically

inhibits DAT function in hDAT-N2A cells, showing a preference for the inhibition of amphetamine-stimulated dopamine efflux as compared to dopamine uptake. Importantly, we demonstrate that the effects of tamoxifen and its metabolites on the DAT occur independently of its activity as selective estrogen receptor modulators. This work suggests that tamoxifen is inhibiting DAT function through a previously unidentified mechanism.

Keywords: amphetamine, dopamine transporter, estrogen receptor, tamoxifen.

J. Neurochem. (2017) **141**, 31–36.

The dopamine transporter (DAT) is a key mechanism by which dopamine is removed from the extracellular space following its release from neurons, and as such plays a vital role in the regulation of dopamine signaling. Pharmacological modulation of the DAT, therefore, is a source of great interest for the treatment of multiple dopaminergic diseases.

The breast cancer therapeutic tamoxifen is a well-established pharmaceutical agent with a clear safety profile; however, tamoxifen has multiple actions beyond its original designation as a selective estrogen receptor modulator (SERM). Various studies have demonstrated that tamoxifen can modulate the dopaminergic system. For example, tamoxifen inhibits amphetamine-stimulated hyperactivity in rodents, potentially through inhibition of protein kinase C (Einat *et al.* 2007). Tamoxifen also has efficacy as a neuroprotectant in models of dopaminergic neurotoxicity (Obata and Kubota 2001; Bourque *et al.* 2007). However, the mechanism by which tamoxifen affects the dopaminergic system remains incompletely elucidated.

Estrogen is well-known to affect DAT function; it stimulates dopamine efflux and enhances amphetamine-stimulated dopamine efflux (Becker 1990; Xiao *et al.* 2003). There are three known estrogen receptors: ER α , ER β , and the G-protein-coupled estrogen receptor (GPER1). All three receptors modulate DAT function to varying

degrees (Alyea *et al.* 2008; Alyea and Watson 2009). Beyond its SERM activity at ER α and ER β , tamoxifen is also an agonist at GPER1 (Thomas *et al.* 2005).

Tamoxifen is metabolized into three active metabolites that reach appreciable levels in the brain: 4-hydroxytamoxifen, *N*-desmethyltamoxifen, and 4-hydroxy-*N*-desmethyltamoxifen (known as endoxifen). 4-Hydroxytamoxifen and endoxifen are considered the active metabolites of tamoxifen, because their potency as SERMs is 100-fold higher than the parent compound (Lien *et al.* 1991). Any combination of these metabolites could be responsible for the effects of tamoxifen on the dopaminergic system.

In this study we hypothesized that tamoxifen would inhibit function of the dopamine transporter. We demonstrate for the

Received October 14, 2016; revised manuscript received January 5, 2017; accepted January 7, 2017.

Address correspondence and reprint requests to Margaret Gnegy, Department of Pharmacology, University of Michigan Medical School, 2220E MSRB III, 1150 West Medical Center Drive, Ann Arbor, MI 48109-5632, USA. E-mail: pgnegy@umich.edu

Abbreviations used: 4OH, 4-hydroxytamoxifen; DA, dopamine; DAT, dopamine transporter; endox, endoxifen; ER, estrogen receptor; GPER1, G-protein-coupled estrogen receptor 1; hDAT, human dopamine transporter; KRH, Krebs Ringer Hepes buffer; PKC, protein kinase C; SEM, standard error of the mean; SERM, selective estrogen receptor modulator; TMX, tamoxifen.

first time that tamoxifen inhibits DAT function through an uncompetitive or mixed mechanism. We also determine that two of tamoxifen's active metabolites, 4-hydroxytamoxifen and endoxifen also inhibit transporter function. Through blockade of the estrogen receptors with selective antagonists, we confirm that tamoxifen and its metabolites inhibit DAT function independently of their actions at any of the estrogen receptors.

Methods

Materials

All chemicals were obtained from Sigma Aldrich with the exception of [³H]dopamine, which was purchased from Perkin Elmer (Waltham, MA, USA), and G36, which was purchased from Tocris (Minneapolis, MN, USA). Tamoxifen citrate, endoxifen, 4-hydroxytamoxifen, and G36 were prepared as a 50 mM stock and fulvestrant as a 10 mM stock in dimethylsulfoxide. Stock solutions were diluted in the buffers appropriate for the assay. Final concentration of dimethylsulfoxide in all solutions ranged from 0.01 to 0.03% and were kept constant across all conditions within an experiment.

Cell culture

N2 mouse neuroblastoma cells stably expressing the human DAT (hDAT-N2a) cells were grown in Optimem reduced serum media (Gibco, Rockville, MD, USA) supplemented with fetal bovine serum (10%), penicillin-streptavidin (1%), and G418 (100 µg/mL; Gibco) at 37°C in 5% CO₂. For experiments involving estrogen receptor antagonists, cells were maintained under the same conditions and in a similar media formulation except that the Optimem was free of phenol-red and was supplemented with a charcoal-stripped bovine serum (kindly provided by Dr. James Rae, University of Michigan) to limit exposure of the cells to exogenous estrogens. For assays measuring dopamine uptake and amphetamine-stimulated dopamine efflux, cells were trypsinized 1–2 days before the experiment and seeded on 24-well plates (50 000–100 000 cells per well); at the time of the experiment, cell density approached confluency with approximately 200,000 cells per well. All experiments were carried out in triplicate and repeated with at least three different cultures of the cells. All experiments were carried out in concordance with the safety guidelines of the University of Michigan.

[³H]Dopamine uptake

Cells were grown in 24-well plates. Media was aspirated from the wells and cells were washed three times with Krebs Ringer HEPES buffer (KRH, pH 7.4, 25 mM HEPES, 125 mM NaCl, 4.8 mM KCl, 1.2 mM KH₂PO₄, 1.3 mM CaCl₂, 1.2 mM MgSO₄, 5.6 mM glucose, 50 µM pargyline, and 50 µM ascorbic acid) before the addition of 400 µL of drug or vehicle in KRH. Vehicle or drugs were present throughout the experiment. All experiments were conducted at 23°C. After a 1-h incubation with vehicle or drugs, 100 µM cocaine was added to non-specific wells, and [³H]dopamine uptake was initiated 10 min later. For the concentration response curves, [³H]dopamine uptake was initiated by the addition of 10 nM

[³H]dopamine supplemented with 300 nM unlabeled dopamine and the reaction was terminated after 10 min. In assays measuring uptake kinetics, dopamine uptake was initiated by 10 nM [³H]dopamine supplemented with 10 nM to 3 µM unlabeled dopamine and the reaction was terminated after 5 min. [³H]Dopamine uptake was terminated by aspiration of the solution followed by three rapid washes with ice cold KRH. Cells in each well were lysed with 400 µL of 2 M perchloric acid and transferred to scintillation vials with 5 mL of Scintiverse Scintillation Cocktail (Fisher Scientific, Pittsburgh, PA, USA) and counted on a Beckman scintillation counter.

[³H]Dopamine efflux

Media was aspirated from wells and cells were washed three times with KRH before the addition of 200 µL of 50 nM [³H]dopamine plus 5 µM unlabeled dopamine in KRH and incubation at 21°C. After 40 min of [³H]dopamine loading, each well was rapidly washed with KRH three times. KRH plus drugs or vehicle were added to each well. A stable baseline was established by removing and replacing the extracellular solution every 10 min for a total of 50 min. Beginning at 50 min, three 10 min fractions were collected with 20 µM amphetamine added during the second fraction. Baseline was defined as the counts per minute in the fraction immediately preceding the addition of amphetamine. Following removal of the final fraction, cells were lysed with 2 M perchloric acid to quantify total remaining dopamine content. Experiments were carried out in triplicate. An additional well was reserved for each treatment condition which received no amphetamine to quantify non-specific dopamine release. Dopamine efflux was quantified as the percentage of the [³H]dopamine content released during the efflux fraction divided by the total [³H]dopamine present in the cells.

Measurement of surface DAT by biotinylation

hDAT-N2A cells were incubated with 3 µM tamoxifen in KRH for 1 h. The amount of cell surface DAT was determined in these cells with biotinylation using previously described method (Guptaroy *et al.* 2009) with minor modifications. Avidin beads (80 µL) were used to pull down biotinylated cell surface proteins from cells lysates (400 µg). Total and biotinylated fraction of DAT was measured by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting using anti-DAT antibody.

Statistical analysis

Statistical analyses were carried out using Graphpad Prism 6 (San Diego, CA, USA). Data are plotted as mean ± the standard error of the mean (SEM). Significance was set at $p < 0.05$. Comparisons between multiple groups were made with one-way analysis of variants (ANOVA), with *post hoc* Dunnett's multiple comparison test. Non-linear regression was used to determine IC₅₀, V_{max}, and K_m values. Calculated K_m and V_{max} values were compared across experiments by a one-way ANOVA. Kinetic data for tamoxifen were also analyzed using Line-weaver Burke analysis in Prism 6. When concentration response curves were compared, comparison of fits in non-linear regression was used to determine whether curves differed from each other. The null hypothesis was that the best-fit parameter for the value did not differ.

Results

Tamoxifen and the active metabolites 4-hydroxytamoxifen and endoxifen inhibit amphetamine-stimulated dopamine efflux and dopamine uptake in hDAT-N2A cells

To characterize the effects of tamoxifen on DAT function, we quantified [^3H]dopamine uptake and amphetamine-stimulated [^3H]dopamine efflux in hDAT-N2A cells following 1-h pre-treatment with the increasing concentrations of tamoxifen (Fig. 1a). One-way repeated measures ANOVA indicated a significant effect of tamoxifen treatment on [^3H]dopamine uptake ($F(5, 20) = 32.74, p < 0.0001$) and efflux ($F(5, 20) = 41.28, p < 0.0001$) compared to vehicle. *Post hoc* Dunnett's multiple comparisons test revealed a significant decrease in dopamine uptake for 3 μM ($p < 0.001$) and 10 μM ($p < 0.0001$) tamoxifen and a significant decrease in dopamine efflux for 0.3 μM ($p < 0.001$), 3 μM ($p < 0.01$), and 10 μM ($p < 0.0001$) tamoxifen compared to vehicle. There was no significant difference between the concentration response curves generated for dopamine uptake and amphetamine-stimulated dopamine efflux in hDAT-N2A cells (Fig. 1a). See Table 1 for IC_{50} values.

The effects of the active metabolites of tamoxifen on the DAT were characterized. *N*-desmethyltamoxifen had no effect on [^3H]dopamine uptake or efflux through the DAT at concentrations up to 3 μM , but was cytotoxic at higher concentrations as measured by PrestoBlue (ThermoFisher, Wayne, MI, USA data not shown). Tamoxifen and the two remaining metabolites, 4-hydroxytamoxifen and endoxifen were not toxic at any of the concentrations tested; therefore our focus was on these three compounds.

In hDAT-N2A cells, a repeated measures one-way ANOVA indicated a significant effect of 4-hydroxytamoxifen treatment on [^3H]dopamine uptake ($F(5, 15) = 33.68, p < 0.0001$) and efflux ($F(5, 10) = 31.63, p < 0.0001$) (Fig. 1b). *Post hoc* Dunnett's multiple comparisons test

Table 1 IC_{50} s for [^3H]dopamine uptake and efflux in human dopamine transporter-N2A cells (95% CI)

IC_{50}	Tamoxifen	4-Hydroxytamoxifen	Endoxifen
Uptake	4.4 μM (3.0–6.3)	3.6 μM (2.5–5.0)	6.7 μM (3.7–12)***
Efflux	4.5 μM (2.7–7.4)	2.6 μM (1.8–3.7)	1.3 μM (0.9–1.9)

*** $p < .001$ versus IC_{50} for efflux.

revealed a significant decrease in [^3H]dopamine uptake for 1 μM ($p < 0.05$), 3 μM ($p < 0.0001$) and 10 μM (0.0001) 4-hydroxytamoxifen and a significant decrease in amphetamine-stimulated [^3H]dopamine efflux for 3 μM ($p < 0.001$), and 10 μM ($p < 0.0001$) 4-hydroxytamoxifen compared to vehicle. The concentration response curves generated by these two datasets were not significantly different from each other.

A one-way repeated measures ANOVA indicated a significant effect of endoxifen treatment on [^3H]dopamine uptake ($F(4, 12) = 8.24, p < 0.01$) and amphetamine-stimulated [^3H]dopamine efflux ($F(4, 12) = 53.63, p < 0.0001$) (Fig. 1c). *Post hoc* Dunnett's multiple comparisons test revealed a significant decrease in [^3H]dopamine uptake at 3 μM ($p < 0.01$) endoxifen and a significant decrease in amphetamine-stimulated [^3H]dopamine efflux at 1 μM ($p < 0.001$) and 3 μM ($p < 0.0001$) endoxifen as compared to vehicle. The concentration response curves generated by these two data sets were significantly different from each other [$F(1,30) = 20.78, (p < 0.0001)$] with endoxifen exhibiting increased potency for the inhibition of [^3H]dopamine efflux compared to uptake (Table 1).

Tamoxifen effect on dopamine uptake kinetics

To further investigate the action of tamoxifen at the DAT, we determined the effects of tamoxifen on [^3H]dopamine uptake kinetics in hDAT-N2A cells. Tamoxifen significantly

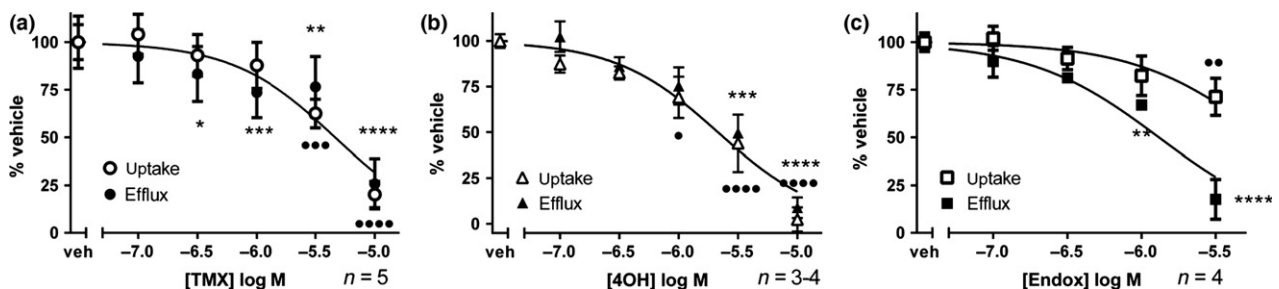


Fig 1 Inhibition of [^3H]dopamine uptake and amphetamine-stimulated [^3H]dopamine efflux in human dopamine transporter-N2A cells following a 1-h treatment with (a) tamoxifen (TMX, $n = 5$), (b) 4-hydroxytamoxifen (4OH, $n = 3-4$), and (c) endoxifen (Endox, $n = 4$). Comparison of fits for non-linear regression indicated no difference between the concentration response curves for uptake versus efflux for

tamoxifen or 4-hydroxytamoxifen, but a significant difference between the concentration response curves for uptake versus efflux for endoxifen. *efflux, $p < 0.05$ versus vehicle; **efflux, $p < 0.01$ versus vehicle; ***efflux, $p < 0.001$ versus vehicle; ****efflux, $p < 0.0001$ versus vehicle; •uptake, $p < 0.05$; ••uptake, $p < 0.01$; •••uptake, $p < 0.001$; ••••uptake, $p < 0.0001$.

decreased the V_{\max} of dopamine uptake at 3 μM and 10 μM . At 10 μM , tamoxifen also significantly decreased the K_m of [^3H]dopamine uptake (Fig. 2a, Table 2). At 3 μM , tamoxifen significantly decreases the V_{\max} of dopamine uptake without having a significant effect on K_m . Lineweaver-Burke analysis (inset, Fig. 2) indicates that the inhibition by 3 μM tamoxifen is uncompetitive, because V_{\max}/K_m for individual vehicle versus 3 μM tamoxifen did not change (0.05 ± 0.004 vs. 0.045 ± 0.005 SEM, respectively). Analysis of inhibition by 10 μM tamoxifen showed mixed inhibition ($V_{\max}/K_m = 0.024 \pm 0.007$, $p < 0.02$ vs. vehicle). The reduction in [^3H]dopamine uptake was not because of a reduction in surface DAT (Fig. 2b). Incubation of hDAT-N2A cells with 3 μM tamoxifen for 1 h had no effect on surface levels of the DAT (2-tailed t -test, $t = 1.020$ $df = 8$, $p = 0.337$).

Effect of estrogen antagonists

N2A mouse neuroblastoma cells express all three known estrogen receptors (Mendez and Garcia-Segura 2006; Manthey *et al.* 2010; Su *et al.* 2012). Because tamoxifen and its metabolites are SERMS, we sought to determine whether their actions on the DAT might be attributable to their activity at any of the three estrogen receptors. The hDAT-N2A cells used in these experiments were maintained in medium supplemented with charcoal stripped fetal bovine serum to preclude complications that might arise from the presence of exogenous estrogens. Using the ER α and ER β antagonist fulvestrant (3 μM), and the GPER1 antagonist G36 (10 μM), we found that blockade of the estrogen receptors had no significant effect on basal [^3H]dopamine

uptake or amphetamine-stimulated [^3H]dopamine efflux. Moreover, neither fulvestrant nor G36 inhibited the effects of tamoxifen or its metabolites on [^3H]dopamine uptake or amphetamine-stimulated [^3H]dopamine efflux (Fig. 3).

Discussion

In this study we demonstrate that tamoxifen and two of its active metabolites, 4-hydroxytamoxifen and endoxifen, impair DAT function independently of their actions at the estrogen receptors. We also demonstrate that in cells, tamoxifen impairs dopamine uptake through a mixed mechanism. This suggests that tamoxifen has a preference for binding to the substrate-occupied transporter indicating an allosteric mechanism of action.

In our investigation of the effects of tamoxifen and its metabolites on the DAT, it was necessary that we consider the potential effects of the drugs' SERM activity on DAT function. We found that antagonists of the estrogen receptors had no effect on amphetamine-stimulated dopamine efflux or dopamine uptake on their own, and did not alter the ability of tamoxifen or its metabolites to inhibit forward or reverse transport. By failing to antagonize the effects of tamoxifen and its metabolites on the DAT, these experiments demonstrated that tamoxifen is not exerting its effects on the DAT through an estrogenic mechanism. Furthermore, because the antagonists themselves had no effect on dopamine uptake or efflux, an antiestrogenic effect of tamoxifen or its metabolites is insufficient to explain its inhibition of DAT function. These conclusions are further supported by previous observations that fulvestrant but not tamoxifen is capable of

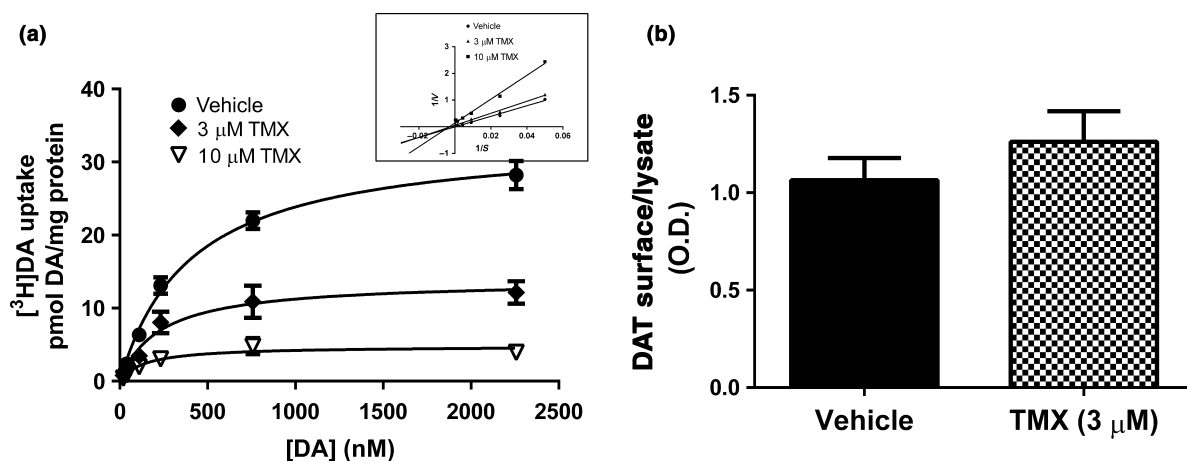


Figure 2 Kinetic analysis of [^3H]dopamine ([^3H]DA) uptake (a) and surface localization of dopamine transporter (DAT) (b) following pretreatment of human DAT (hDAT)-N2A cells with tamoxifen. (a) hDAT-N2A cells were treated with vehicle, 3 μM , or 10 μM tamoxifen (TMX). [^3H]DA uptake was measured for 5 min as described in Methods. Comparison of fits indicated a significant effect of both

concentrations of tamoxifen on V_{\max} but not K_m compared to vehicle ($p < 0.0001$). (a, inset) Lineweaver-Burke plot of the data. Data are represented as mean \pm SEM. (b) Surface DAT was measured by biotinylation following a 1 h incubation with vehicle or 3 μM tamoxifen as described in Methods. $N = 5$.

Table 2 Best-fit Michaelis–Menten values for [³H]dopamine uptake

Best-fit values	Vehicle (n = 6)	3 μM TMX (n = 3)	10 μM TMX (n = 3)
V _{max} (pmoles/5 min/mg ± SEM)	34.5 ± 2.8	13.8 ± 2.1**	4.9 ± 0.9****
K _m (nM ± SEM)	430.9 ± 57.2	232.6 ± 33.7	148.6 ± 53.9*

*p < 0.05, **p < 0.01, ****p < 0.0001 versus vehicle, one-way ANOVA with Dunnett's *post hoc* multiple comparisons.

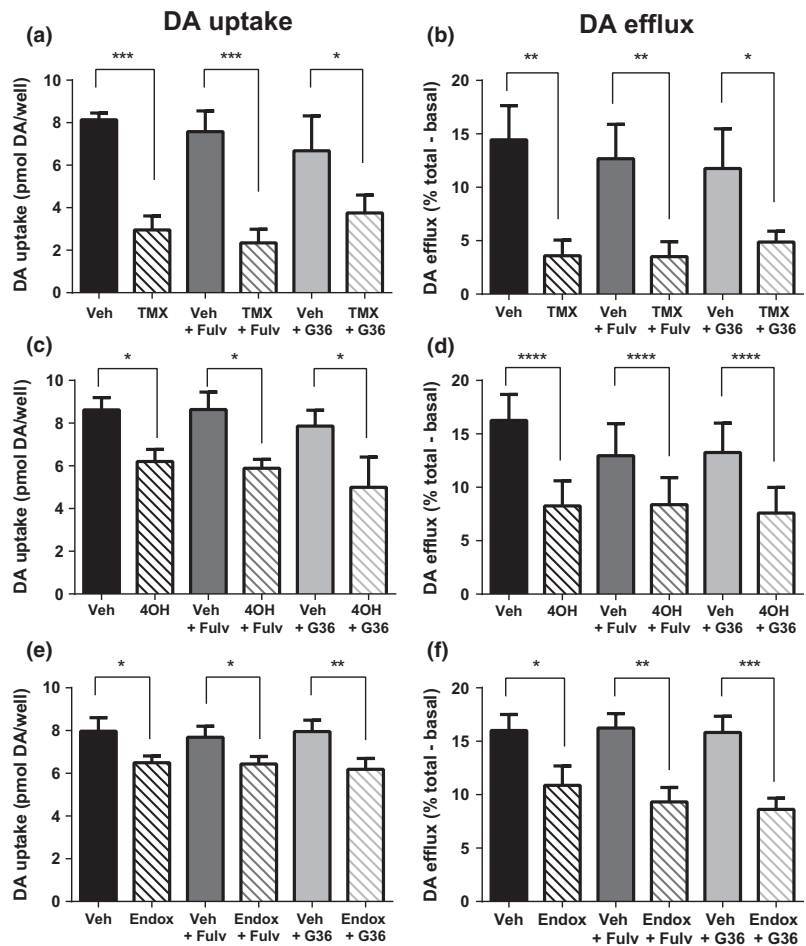


Figure 3 Effects of estrogen receptor antagonists on [³H]dopamine (DA) uptake and amphetamine-stimulated [³H]dopamine efflux. Inhibition of [³H]dopamine uptake (a, c and e) and amphetamine-stimulated [³H]dopamine efflux (20 μM amphetamine, b, d and f) by tamoxifen (10 μM, a and b), 4-hydroxytamoxifen (3 μM, c and d), and endoxifen (3 μM, e and f) were measured in the presence and absence of the estrogen receptor (ER) α /ER β antagonist fulvestrant (3 μM) or the G-protein coupled estrogen receptor 1 (GPER1) antagonist G36 (10 μM). The presence of the estrogen receptor antagonists had no significant impact on amphetamine-stimulated [³H]dopamine efflux or [³H]dopamine uptake and failed to affect the ability of tamoxifen and its metabolites to inhibit these activities. *p < 0.05, **p < 0.01, ***p < 0.001 as compared to vehicle control, 2-tailed paired t test. N = 3.

antagonizing the effect of estrogen on amphetamine-stimulated efflux (Xiao *et al.* 2003). In addition, endoxifen and 4-hydroxytamoxifen are 100-fold more potent in their actions at the estrogen receptors, relative to tamoxifen (Lien *et al.* 1991), but all of the compounds tested display IC₅₀s in the micromolar range for reduction in DAT function. The diphenyl structural motif in tamoxifen and its metabolites could partially account for their blockade of dopamine uptake; this motif also exists in DAT blockers such as modafinil and benzotropine (Schmitt *et al.* 2013).

Though tamoxifen and 4-hydroxytamoxifen exhibit similar effects on uptake and efflux our results indicate that endoxifen preferentially inhibits dopamine efflux in the cells. The difference between the effects of endoxifen on

dopamine uptake and amphetamine-stimulated dopamine efflux in the cells is intriguing. In the cell-based assay in which [³H]dopamine is exogenously loaded, the [³H]-dopamine measured is actually the difference between the outward transport of dopamine and the amount of dopamine taken back up in to the cell during assay. Thus an inhibitor of dopamine uptake might be expected to display less of an effect on amphetamine-stimulated dopamine efflux, because the blockade of reuptake might obfuscate a reduction in efflux. This was not true of endoxifen, however. Another activity of endoxifen might play a role in the enhanced potency for reverse transport. Tamoxifen and its metabolites, including endoxifen, inhibit protein kinase C activity (O’Brian *et al.* 1985). In cell-based assays,

tamoxifen exhibits micromolar potency in the inhibition of phorbol myristate acetate-stimulated phosphorylation of protein kinase C (PKC) substrates (unpublished data). Our previous studies (Kantor and Gnegy 1998; Johnson *et al.* 2005) have shown that protein kinase C inhibitors reduce amphetamine-stimulated dopamine efflux but do not inhibit dopamine reuptake. Thus, at the concentrations tested here, it is reasonable to expect that tamoxifen and its metabolites are inhibiting PKC, although this may not contribute to the reduction in dopamine uptake. It is possible that the enhanced effectiveness of endoxifen in reducing dopamine efflux is because of a PKC-dependent inhibition of amphetamine-stimulated dopamine efflux, however. Reportedly, endoxifen is more potent at inhibiting PKC than is tamoxifen (Ali *et al.* 2010).

This work demonstrates for the first time that tamoxifen inhibits function of the DAT independently of its actions as a selective estrogen receptor modulator. Future work will aim to better characterize the interaction between the DAT and tamoxifen and clarify a mechanism of action for these observations.

Acknowledgments and conflict of interest disclosure

Funding support comes from R01DA11697 (MEG), T32DA007267 and T32GM007767 (SRM). The authors have no conflicts of interest to declare.

References

- Ali S. M., Ahmad A., Shahabuddin S., Ahmad M. U., Sheikh S. and Ahmad I. (2010) Endoxifen is a new potent inhibitor of PKC: a potential therapeutic agent for bipolar disorder. *Bioorg. Med. Chem. Lett.* **20**, 2665–2667.
- Alyea R. A. and Watson C. S. (2009) Nongenomic mechanisms of physiological estrogen-mediated dopamine efflux. *BMC Neurosci.* **10**, 59.
- Alyea R. A., Laurence S. E., Kim S. H., Katzenellenbogen B. S., Katzenellenbogen J. A. and Watson C. S. (2008) The roles of membrane estrogen receptor subtypes in modulating dopamine transporters in PC-12 cells. *J. Neurochem.* **106**, 1525–1533.
- Becker J. B. (1990) Direct effect of 17 beta-estradiol on striatum: sex differences in dopamine release. *Synapse* **5**, 157–164.
- Bourque M., Liu B., Dluzen D. E. and Di Paolo T. (2007) Tamoxifen protects male mice nigrostriatal dopamine against methamphetamine-induced toxicity. *Biochem. Pharmacol.* **74**, 1413–1423.
- Einat H., Yuan P., Szabo S. T., Dogra S. and Manji H. K. (2007) Protein kinase C inhibition by tamoxifen antagonizes manic-like behavior in rats: implications for the development of novel therapeutics for bipolar disorder. *Neuropsychobiology* **55**, 123–131.
- Guptaroy B., Zhang M., Bowton E., Binda F., Shi L., Weinstein H., Galli A., Javitch J. A., Neubig R. R. and Gnegy M. E. (2009) A juxtamembrane mutation in the N-terminus of the dopamine transporter induces preference for an inward-facing conformation. *Mol. Pharmacol.* **75**, 514–524.
- Johnson L. A., Guptaroy B., Lund D., Shamban S. and Gnegy M. E. (2005) Regulation of amphetamine-stimulated dopamine efflux by protein kinase C beta. *J. Biol. Chem.* **280**, 10914–10919.
- Kantor L. and Gnegy M. E. (1998) Protein kinase C inhibitors block amphetamine-mediated dopamine release in rat striatal slices. *J. Pharmacol. Exp. Ther.* **284**, 592–598.
- Lien E. A., Solheim E. and Ueland P. M. (1991) Distribution of tamoxifen and its metabolites in rat and human tissues during steady-state treatment. *Cancer Res.* **51**, 4837–4844.
- Manthey D., Gamerding M. and Behl C. (2010) The selective beta1-adrenoceptor antagonist nebivolol is a potential oestrogen receptor agonist with neuroprotective abilities. *Br. J. Pharmacol.* **159**, 1264–1273.
- Mendez P. and Garcia-Segura L. M. (2006) Phosphatidylinositol 3-kinase and glycogen synthase kinase 3 regulate estrogen receptor-mediated transcription in neuronal cells. *Endocrinology* **147**, 3027–3039.
- Obata T. and Kubota S. (2001) Protective effect of tamoxifen on 1-methyl-4-phenylpyridine-induced hydroxyl radical generation in the rat striatum. *Neurosci. Lett.* **308**, 87–90.
- O'Brian C. A., Liskamp R. M., Solomon D. H. and Weinstein I. B. (1985) Inhibition of protein kinase C by tamoxifen. *Cancer Res.* **45**, 2462–2465.
- Schmitt K. C., Rothman R. B. and Reith M. E. (2013) Nonclassical pharmacology of the dopamine transporter: atypical inhibitors, allosteric modulators, and partial substrates. *J. Pharmacol. Exp. Ther.* **346**, 2–10.
- Su C., Rybalchenko N., Schreihof D. A., Singh M., Abbassi B. and Cunningham R. L. (2012) Cell Models for the Study of Sex Steroid Hormone Neurobiology. *J. Steroids Horm Sci* **S2**, 003.
- Thomas P., Pang Y., Filardo E. J. and Dong J. (2005) Identity of an estrogen membrane receptor coupled to a G protein in human breast cancer cells. *Endocrinology* **146**, 624–632.
- Xiao L., Jackson L. R. and Becker J. B. (2003) The effect of estradiol in the striatum is blocked by ICI 182,780 but not tamoxifen: pharmacological and behavioral evidence. *Neuroendocrinology* **77**, 239–245.